Separation of phenylalanine, tyrosine and tryptophan by chromatography on poly-N-vinyl pyrrolidone

We have recently reported on the use of poly-N-vinyl pyrrolidone (PVP) column chromatography for the fractionation[1] and desalting[2] of nucleotides, nucleosides, purines and pyrimidines. PVP has now been found effective for the separation of the aromatic amino acids phenylalanine, tyrosine and tryptophan.

Materials and methods

L-Tyrosine, L-tryptophan and L-phenylalanine were purchased from Sigma Chemical Co*. Insoluble PVP, sold under the trade name of Polyclar AT Powder, was obtained from GAF Corporation, New York.

The Polyclar AT was mixed with distilled water, and the fines were discarded by repeated decantation.

A 1 ml water solution containing phenylalanine (1.6 mg), tyrosine (0.8 mg) and tryptophan (0.38 mg) was applied to a 1.0 × 40.5 cm column and elution was carried out with distilled water (pH 6) at a flow rate of 18 ml/h at room temperature and atmospheric pressure. The effluent (collected in 1.2 ml fractions) was monitored at 256 mμ for phenylalanine and 275 mμ for tyrosine and tryptophan in a Gilford 220 spectrophotometer. The U.V.-absorbing compounds eluted were identified by reference to known spectra of the three aromatic amino acids.

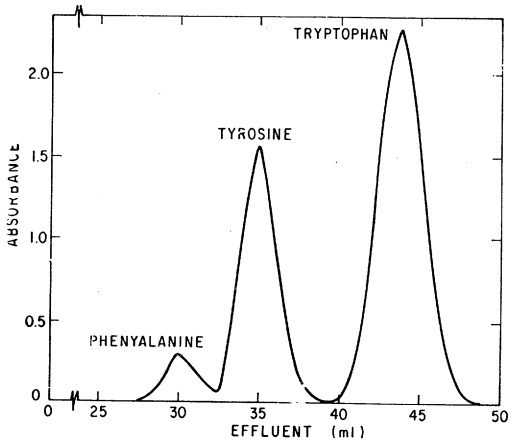

Fig. 1. Fractionation of phenylalanine, tyrosine and tryptophan on a 1.0 × 40.5 cm column. Phenylalanine (1.6 mg), tyrosine (0.8 mg) and tryptophan (0.38 mg) in 1 ml water, eluted with distilled water at a flow rate of 18 ml/h. Absorbance measurements at 275 mμ except for phenylalanine at 256 mμ.

Results and discussion

Fig. 1 shows excellent resolution of the three components in 2.5 h. The amino acids are recovered in small volumes using only water for elution.

* Mention of trade or company names does not imply endorsement by the Department over others not named.

Previous publications[1,3,4] have indicated that hydrogen bonding is the principal mechanism involved in binding to PVP. The adsorption of these amino acids appears to adhere to this concept.

Based on previous work[2], we would expect salts to elute earlier than tyrosine and tryptophan. Thus PVP may also be useful in the desalting of these two amino acids.

PVP chromatography may provide a convenient approach to the separation of phenylalanine, tyrosine and tryptophan from complex mixtures such as those containing polymeric plant pigments.

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